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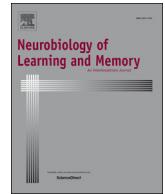
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A brief period of sleep deprivation causes spine loss in the dentate gyrus of mice

Frank Raven^a, Peter Meerlo^a, Eddy A. Van der Zee^a, Ted Abel^b, Robbert Havekes^{a,*}

^a Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, The Netherlands

^b Department of Molecular Physiology and Biophysics, Iowa Neuroscience Institute, University of Iowa, Iowa City, Iowa, USA

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ABSTRACT

Sleep and sleep loss have a profound impact on hippocampal function, leading to memory impairments. Modifications in the strength of synaptic connections directly influences neuronal communication, which is vital for normal brain function, as well as the processing and storage of information. In a recently published study, we found that as little as five hours of sleep deprivation impaired hippocampus-dependent memory consolidation, which was accompanied by a reduction in dendritic spine numbers in hippocampal area CA1. Surprisingly, loss of sleep did not alter the spine density of CA3 neurons. Although sleep deprivation has been reported to affect the function of the dentate gyrus, it is unclear whether a brief period of sleep deprivation impacts spine density in this region. Here, we investigated the impact of a brief period of sleep deprivation on dendritic structure in the dentate gyrus of the dorsal hippocampus. We found that five hours of sleep loss reduces spine density in the dentate gyrus with a prominent effect on branched spines. Interestingly, the inferior blade of the dentate gyrus seems to be more vulnerable in terms of spine loss than the superior blade. This decrease in spine density predominantly in the inferior blade of the dentate gyrus may contribute to the memory deficits observed after sleep loss, as structural reorganization of synaptic networks in this subregion is fundamental for cognitive processes.

1. Introduction

Sleep is a universal phenomenon, but its function remains one of the most fundamental questions in life sciences. It is becoming an even more pressing matter as sleep shortage is a growing major public health issue due to work schedules and around-the-clock lifestyles that allow too little time for recovery. Repeated loss of sleep has severe consequences for brain function, performance, and overall wellbeing (Bryant, Trinder, & Curtis, 2004; Harrison and Horne, 2000; Krause et al., 2017). Furthermore, chronic sleep loss has been recognized as a risk factor for various disorders such as psychiatric disorders and can even have fatal consequences in a matter of months or years (Meerlo, Havekes, & Steiger, 2015).

Substantial evidence derived from both human and animal research indicates that even a short period of sleep deprivation (SD) can negatively impact brain function, including attention, decision making and various types of memory (Abel, Havekes, Saletin, & Walker, 2013; Havekes, Meerlo, & Abel, 2015; Walker, 2008). Interestingly, recent studies investigating specific types of memory revealed that the

hippocampus is especially vulnerable to the negative consequences of sleep loss (Abel et al., 2013; Havekes and Abel, 2017; Kreutzmann, Havekes, Abel, & Meerlo, 2015; Saletin et al., 2016). For example, even a single night of SD has been shown to impair hippocampus-dependent memory consolidation in humans (Van Der Werf et al., 2009; Yoo, Hu, Gujar, Jolesz, & Walker, 2007). Likewise, rodent studies investigating the link between sleep and hippocampus-dependent memory consolidation showed that a brief 5–6 h period of SD disrupts the consolidation of contextual fear memories, without affecting hippocampus-independent forms of fear memories (Graves, Heller, Pack, & Abel, 2003; Hagerwood et al., 2010; Hagerwood, Bultsma, Barf, Koolhaas, & Meerlo, 2011; Havekes et al., 2015; Vecsey et al., 2009). Furthermore, object-location memories, which also require the hippocampus (Oliveira, Hawk, Abel, & Havekes, 2010), are similarly affected by 5–6 h of SD directly following training (Havekes et al., 2014, 2016; Prince et al., 2014; Tudor et al., 2016). Hence, memory processes that require the hippocampus seem to be particularly sensitive to sleep loss.

Information flows into the hippocampus from neurons of the entorhinal cortex that project through the perforant pathway onto the

Abbreviations: BDNF, brain-derived neurotrophic factor; DG, Dentate Gyrus; EGFP, enhanced green fluorescent protein; LTP, long-term potentiation; NMDA, N-methyl-d-aspartate; NSD, non-sleep deprived; pCREB, phosphorylated cAMP response element-binding protein; PDE, phosphodiesterase; REM, rapid-eye movement; SD, sleep deprivation

* Corresponding author at: Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen 9747 AG, The Netherlands.

E-mail address: r.havekes@rug.nl (R. Havekes).

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granule cells of the dentate gyrus (DG) (Amaral, 1993; Andersen, Bliss, & Skrede, 1971; Nguyen and Kandel, 1996). The granule cells then send their axons, also known as the mossy fibers, to the pyramidal cells of the CA3 area. Subsequently, CA3 pyramidal cell axons or Schaffer collaterals project to pyramidal cells in the CA1 area. The information from CA1 terminates in the subiculum and then on to deeper layers of the entorhinal cortex (Amaral, 1993; Andersen et al., 1971; Nguyen and Kandel, 1996). It is important to note that both CA3 and CA1 neurons receive direct input from cortical layers as well. Because the DG filters information that enters the hippocampus (Havekes and Abel, 2009), it has been suggested to play a key role in learning, memory and spatial coding (Aimone, Deng, & Gage, 2011). In agreement with this is the finding that DG lesions in rats cause an impairment in spatial learning (Gilbert, Kesner, & Lee, 2001; Kesner, 2017). In addition, a mutant mouse line, which lacks the essential subunit of the N-methyl-D-aspartate (NMDA) receptor NR1 specifically in DG granule cells, was not able to differentiate between two similar contexts, indicating an impairment in pattern separation (McHugh et al., 2007), reviewed in (Havekes and Abel, 2009). Thus, it is clear that the DG plays an important role in memory processes.

Long-term potentiation (LTP) is a cellular model for memory storage that has been used to study the impact of SD on specific hippocampal circuits. A long period (i.e., 24 h or longer) of rapid-eye movement (REM) SD impairs long-term memory and LTP in the CA1 area of the hippocampus (McDermott et al., 2003; Zagaar, Dao, Levine, Alhaider, & Alkadhi, 2013). Furthermore, even a brief period (5–6 h) of total SD affects long-lasting forms of LTP in the CA1 area (Havekes et al., 2016; Vecsey et al., 2009). In addition, LTP is studied not only in the CA1 area, but also in the DG. Long-term REM sleep restriction for 21 days (18 h a day) using the multiple platforms method attenuates LTP in the DG (Suer et al., 2011) and such SD-induced impairments can be prevented by both chronic caffeine treatment and regular exercise (Alhaider and Alkadhi, 2015; Zagaar, Dao, Alhaider, & Alkadhi, 2016). Importantly, even acute REM SD for 24 h or 3–4 h of total SD already decreases LTP in the granule cells of the DG (Marks and Wayner, 2005; Romcy-Pereira and Pavlides, 2004). Thus, synaptic plasticity within specific subregions of the hippocampus, such as the DG, are highly sensitive to sleep loss.

Synaptic plasticity has been closely linked to the formation, maintenance, and elimination of dendritic spines (De Roo, Klauser, Garcia, Poglia, & Muller, 2008; Lamprecht, 2014; Lynch, 2004; van der Zee, 2015). For example, LTP induction caused changes in spine morphology including the enlargement of the spine head, as well as the widening of the spine neck (Bourne and Harris, 2008; Raven, Van der Zee, Meerlo, & Havekes, 2017; Yuste and Bonhoeffer, 2001). In contrast to LTP, long-term depression, characterized by a long-lasting decrease in synaptic transmission, induces spine shrinkage and a decrease in spine numbers (Bourne and Harris, 2008; Hasegawa, Sakuragi, Tominaga-Yoshino, & Ogura, 2015; Zhou, Homma, & Poo, 2004). Such spine dynamics are thought to be crucial for the storage of new information and memory consolidation (De Roo et al., 2008; Lamprecht and LeDoux, 2004; Lamprecht, 2014; Raven et al., 2017) and an impairment of spine dynamics may be an important mechanism of SD-induced memory impairments (Areal, Warby, & Mongrain, 2017; Havekes and Abel, 2017; Puentes-Mestral and Aton, 2017; Raven et al., 2017). For instance, sleep-dependent motor learning has recently been associated with changes in spine dynamics in the motor cortex (Li, Ma, Yang, & Gan, 2017). Furthermore, 5–6 h of total SD leads to a reduction of spine density of all subtypes in the CA1 region of the hippocampus (Havekes et al., 2016). The loss of spines was causally linked to the impairments in LTP in the hippocampal Schaffer collaterals, as preventing the spine loss in the sleep-deprived hippocampus made LTP and memory resilient to the negative impact of prolonged wakefulness (Havekes et al., 2016). Strikingly, such changes were absent in area CA3 of the hippocampus (Havekes et al., 2016). The latter observation suggests that CA1 neurons are more vulnerable to the consequences of sleep loss than CA3

neurons at the level of structural plasticity. Despite the reports that SD attenuates LTP in the DG (Marks and Wayner, 2005; Romcy-Pereira and Pavlides, 2004), no studies have examined whether a brief period of SD affects structural plasticity in this region. Therefore, in the present study, we examined the impact of 5 h of SD on spine density in the DG.

2. Materials and methods

2.1. Subjects

Three month old male C57BL/6J mice were obtained from Jackson Laboratories and housed in groups of 4 with littermates. Animals were housed on a 12h light: 12h dark schedule with lights on at 7 am (ZT 0). Mice had food and water available *ad libitum* and one week prior to the SD experiment mice were single housed. All experiments were conducted according to National Institutes of Health guidelines for animal care and were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

2.2. Sleep deprivation

All mice were handled for two minutes on five consecutive days prior to the SD experiment in order to habituate them to the experimenter without affecting synaptic plasticity (Vecsey et al., 2013). After the habituation phase, the mice were randomly assigned to the control or SD group. The animals of the SD group were sleep deprived for five hours using the gentle stimulation method as described in our previously published papers (Havekes et al., 2014, 2016; Prince and Abel, 2013; Tudor et al., 2016). In short, animals were kept awake by gently tapping the cage, gently shaking the cage, and/or removing the wire cage top. Their bedding was disturbed only in cases when mice did not respond to tapping or shaking the cage. Importantly, we did not use any novel objects, cages or other arousing stimuli to keep the animals awake. This method of SD has been validated by our laboratory using EEG recordings (Meerlo, de Bruin, Strijkstra, & Daan, 2001). Furthermore, several studies have shown that the cognitive deficits and synaptic plasticity impairments as a result of SD were not caused by elevated plasma corticosterone levels or the gentle stimulation method itself (Hagewoud et al., 2010; Meerlo and Turek, 2001; Ruskin, Dunn, Billiot, Bazan, & LaHoste, 2006; Tiba, Oliveira, Rossi, Tufik, & Suchecki, 2008; van der Borgh et al., 2006; Vecsey et al., 2009). The role of glucocorticoids in synaptic plasticity and memory deficits associated with SD has been extensively discussed previously (Havekes et al., 2015; Kreutzmann et al., 2015) and briefly in the discussion of the present paper.

2.3. Golgi analyses

Brains were impregnated using the Rapid Golgi stain kit (FD Neurotechnologies Inc., Columbia, MD, USA) according to the instructions and described previously (Havekes et al., 2016). Coronal sections (80- μ m thickness) that covered the rostro-caudal axis of DG of the hippocampus were analyzed. The serial sections were chosen and analyzed using a stereology-based software (NeuroLucida, v10, MicroBrightfield, Williston, VT, USA) and a Zeiss Axioplan 2 image microscope with an Optronics MicroFire CCD (1600 \times 1200) digital camera, motorized X, Y, and Z-focus for high-resolution image acquisition and digital quantitation. In combination with a 100x objective, using a sophisticated and well-established method, this should represent a 3D quantitative profile of the neurons sampled and prevent a failure to detect less prominent spines. Analyses were performed blindly by Neurodigitech (San Diego, CA, USA).

Our sampling strategy was to prescreen the impregnated neurons along the anterior/posterior axis of the region of interest to see if they were qualified for analysis. Neurons with incomplete impregnation or neurons with truncations due to the plane of sectioning were not

analyzed. Moreover, cells with dendrites labeled retrogradely by impregnation in the surrounding neuropil were excluded as well. We also made sure there was a minimal level of truncation at the most distal part of the dendrites; this often happens in most of the Golgi studies, likely due to the plane of sectioning at top and bottom parts of the section. With consideration of the shrinkage factor after processing (generally 10–25% shrinkage), the visualization of the spine subclass is no issue as we used a 100x Zeiss objective lens with immersion oil, which is sufficient to resolve the details or subtype of the spines for laborious counting.

2.4. Statistics

All Golgi analyses were conducted by an experimenter blind to treatment. Data sets were analyzed using non-paired t-tests. Differences were considered statistically significant when $p < 0.05$. All data are plotted as mean \pm SEM.

3. Results

3.1. Sleep deprivation attenuates spine density in the dentate gyrus

To study the effect of SD on the structural plasticity within the DG, we first investigated the effect of SD on spine density within the DG as a whole. Sleep deprivation reduced the spine density of basal dendrites of dentate granule cells ($p < 0.05$, Figs. 1 and 2A). Subsequently, we examined whether SD has a more profound effect on either of the two blades of the dentate gyrus. SD decreased spine density in the inferior blade ($p < 0.05$, Fig. 2B), whereas there was a minor, non-significant effect on the granule cells in the superior blade of the DG ($p > 0.1$, Fig. 2C). We found no effect of SD on spine length, even when we differentiated between the two blades of the DG. Altogether, sleep loss appears to reduce spine density, targeting predominantly the inferior blade of the DG.

3.2. Sleep deprivation reduces spine density of specific spine subtypes in the dentate gyrus

To examine the effects of sleep loss on the DG in more detail, we assessed whether SD affects specific spine subtypes in the DG. The density of both thin and branched spines was reduced in the DG by SD ($p < 0.05$, Fig. 3A). Separate analyses of the two blades revealed that the density of thin and branched spines was reduced in the inferior blade ($p < 0.05$, Fig. 3B). In contrast, only the density of branched spines was decreased in the superior blade of the dentate gyrus

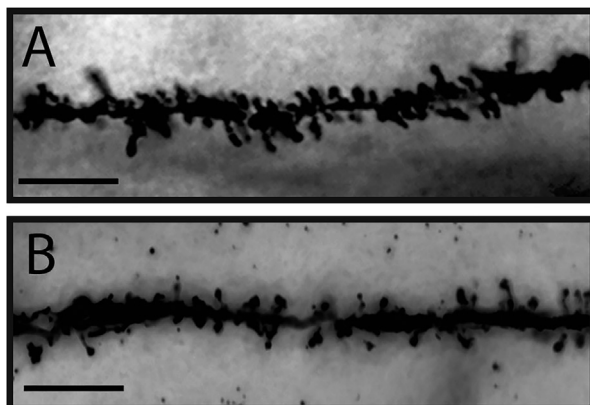


Fig. 1. Sleep deprivation reduces the spine density in dentate granule cells of the hippocampus. Representative images of Golgi-impregnated dendritic spines of dentate granule cells from sleep deprived (SD) and non-sleep deprived (NSD) mice. SD mice (bottom panel) showed a significant reduction in spines compared to NSD mice (upper panel). Scale bar, 5 μ m.

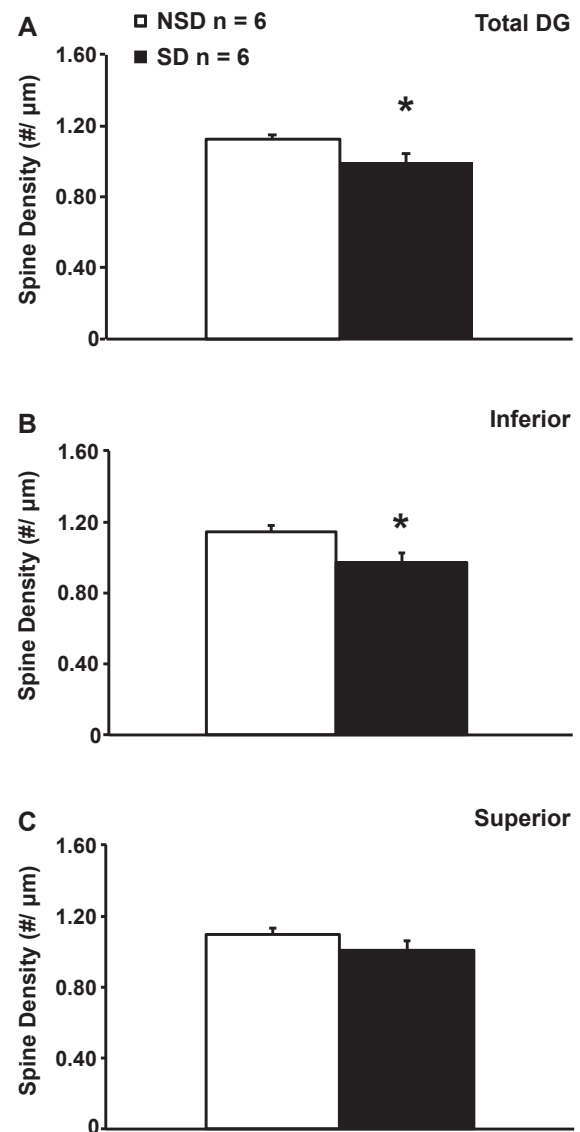


Fig. 2. Sleep deprivation predominantly affects the spine density in the inferior blade of the dentate gyrus. (A) Sleep deprivation decreases the total spine density of basal dendrites of DG neurons ($n = 6$, Student's t -test, $p = 0.038$). (B, C) Sleep deprivation attenuates spine numbers in the inferior blade of the dentate gyrus ($n = 6$, Student's t -test, $p = 0.043$) without affecting spine numbers in the superior blade of the dentate gyrus ($n = 6$, Student's t -test, $p = 0.202$). NSD: non-sleep deprived, SD: sleep deprived. Values represent the mean \pm SEM. * $p < 0.05$, by Student's t -test.

($p < 0.05$, Fig. 3C). These observations indicate that specific spine subtypes are affected by SD, and that the impact of SD affects the DG in a blade-specific fashion.

3.3. Sleep deprivation decreases spine density in the first few orders of dentate granule cells

To identify whether the spine changes occur at specific branches, we analyzed branch one to six of the dendritic tree. Branch-specific analyses indicated that SD reduced the spine density from the first to fourth branch in the DG ($p < 0.05$, Fig. 4A). In the inferior blade, SD strongly attenuated spine density from the first to third branch orders ($p < 0.005$, Fig. 4B), with a trend towards a decrease of spine density at branch four ($p < 0.1$, Fig. 4B). Comparable findings were found in the superior blade of the DG, as spine density was also reduced from the first to third branch orders of dentate granule cells in the superior blade

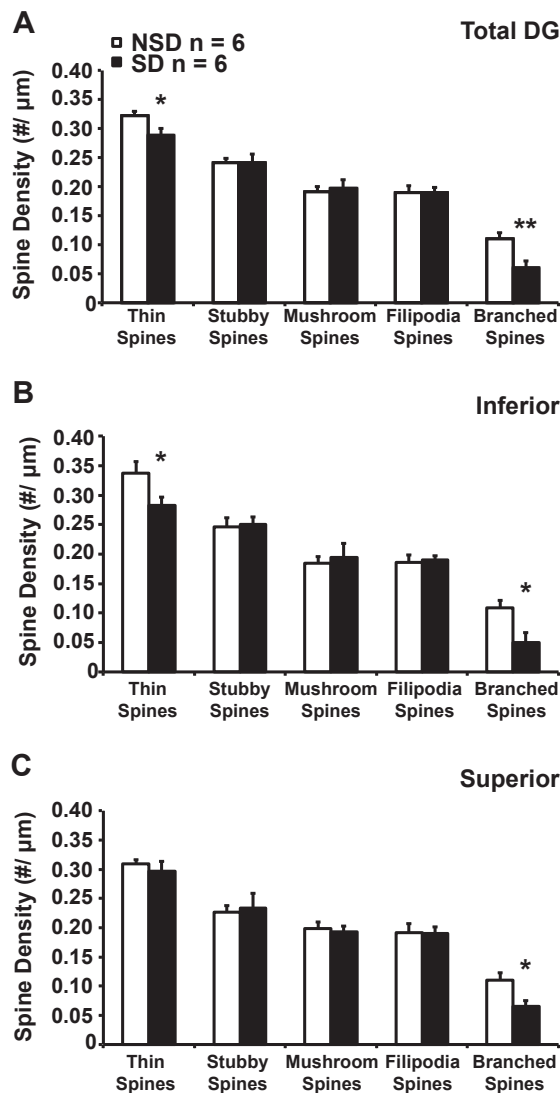


Fig. 3. Sleep deprivation attenuates the specific spine-subtypes in the dentate granule cells of the hippocampus. (A) Sleep deprivation reduces the total number of thin and branched spines in the dendrites of dentate granule cells ($n = 6$, Student's t -test, * $p < 0.05$, ** $p < 0.01$). (B) Sleep deprivation attenuates the number of thin and branched spines in the dendrites of dentate granule cells in the inferior blade of the dentate gyrus ($n = 6$, Student's t -test, $p < 0.05$). (C) Sleep deprivation specifically decreases the number of branched spines in the dendrites of dentate granule cells in the superior blade of the dentate gyrus ($n = 6$, Student's t -test, $p < 0.05$). NSD: non-sleep deprived, SD: sleep deprived. Values represent the mean \pm SEM. * $p < 0.05$, by Student's t -test.

($p < 0.05$, Fig. 4C). Thus, these results further support the finding that SD targets spine density in dentate granule cells, and predominantly in the inferior blade.

3.4. Sleep deprivation reduces spine density at specific distances from the soma of dentate granule cells

We previously showed that SD affects CA1 spine density at specific distances (Havekes et al., 2016). Therefore, we also investigated whether SD exerts its effect at specific distances from the soma in granule cells. Indeed, SD reduced spine density of dendrites at 30, 60, 120, and 150 μ m distance from the soma of dentate granule cells ($p < 0.05$, Fig. 5A). Remarkably, SD resulted in an increase of spine density at 240 μ m from soma ($p < 0.05$, Fig. 5A). In the inferior blade, SD

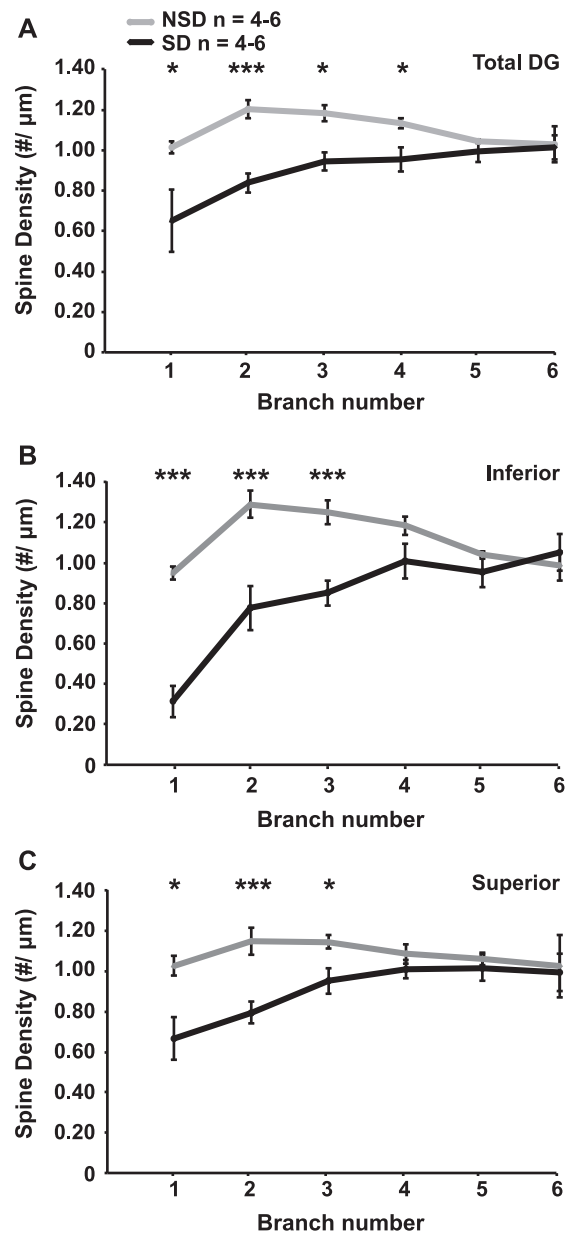


Fig. 4. Sleep deprivation attenuates the spine density at specific branch numbers of dentate granule cells. (A) Sleep deprivation reduces the density of dendritic spines at branch number 1–4 of the dentate granule cells ($n = 5–6$, Student's t -test, * $p < 0.05$, *** $p < 0.005$). (B) Sleep deprivation reduces the spine density at branch number 1–4 in the inferior blade of the dentate granule cells ($n = 5–6$, Student's t -test, $p < 0.005$). (C) Sleep deprivation reduces the spine density at branch number 1–3 in the superior blade of the dentate gyrus ($n = 5–6$, Student's t -test, * $p < 0.05$, *** $p < 0.005$). NSD: non-sleep deprived, SD: sleep deprived. Values represent the mean \pm SEM. * $p < 0.05$, *** $p < 0.005$, by Student's t -test.

reduced spine density at 30, 60, 120, and 150 μ m away from the soma ($p < 0.05$, Fig. 5B). However, SD decreased spine density at 30, 120, and 150 μ m from soma in the superior blade ($p < 0.05$, Fig. 5C), indicating that the spines at 60 μ m from soma were spared in the superior blade of the DG. Notably, only a trend towards an increase was found at 240 μ m when the two blades were analyzed separately ($p > 0.05$, Fig. 5B and C). Altogether, SD impacts spine density of dentate granule cells and especially the regions corresponding to the beginning and middle range of the dendritic branch. Furthermore, especially the inferior blade of the DG seems to be sensitive to sleep loss.

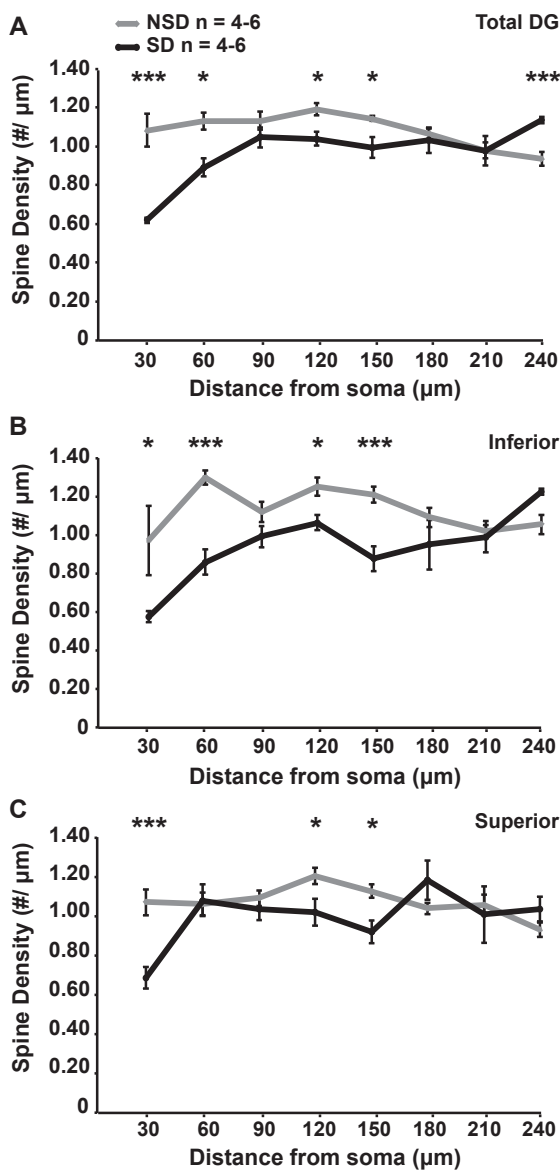


Fig. 5. Sleep deprivation decreases the spine density of basal dendrites at specific distances from soma of dentate granule cells. (A) Sleep deprivation reduces the total spine density of dendrites at 30, 60, 120 and 150 μm from the soma, while it caused an increase in spine density at 240 μm from the soma ($n = 5-6$, Student's t -test, * $p < 0.05$, *** $p < 0.005$). (B) Sleep deprivation reduces spine density at 30, 60, 120 and 150 μm from the soma of dentate granule cells in the inferior blade ($n = 5-6$, Student's t -test, * $p < 0.05$, *** $p < 0.005$). (C) Sleep deprivation attenuates the spine density at 30, 120 and 150 μm away from the soma of dentate granule cells in the superior blade ($n = 5-6$, Student's t -test, * $p < 0.05$, *** $p < 0.005$). NSD: non-sleep deprived, SD: sleep deprived. Values represent the mean \pm SEM. * $p < 0.05$, *** $p < 0.005$, by Student's t -test.

4. Discussion

This study demonstrated that in mice 5 h of SD leads to a reduction of spine density in the DG, particularly in the inferior blade of the DG. Furthermore, SD resulted in a decreased density of specific spine subtypes, without affecting dendrite length. While SD led to a reduction of both branched and thin spines in the inferior blade, only the density of branched spines was reduced in the superior blade. In addition, as reflected by the analysis of branch number and distance from soma, the effect of SD on spine density seems most prominent in the first few branches of the dendritic tree in the DG. This effect could be observed

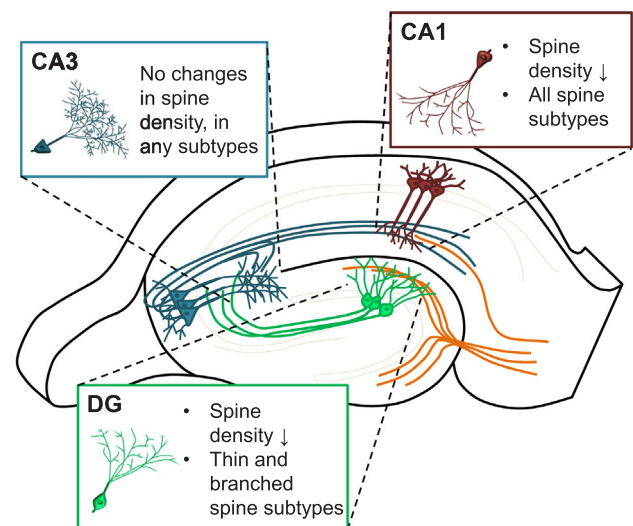


Fig. 6. Sleep deprivation affects specific subregions of the hippocampus. Subregion-specific spine loss as a result of sleep deprivation was observed in the dentate gyrus and CA1 region of the hippocampus. In contrast spine numbers in area CA3 were not affected by a brief period of sleep deprivation. Data from area CA1 and CA3 were previously published (Havekes et al., 2016).

in both blades of the DG, although the impact of SD appeared to be more pronounced in the inferior blade of the DG. Other studies assessing the impact of sleep loss on structural plasticity in the hippocampus found that SD affects the CA1 region (Acosta-Pena et al., 2015; Havekes et al., 2016). In contrast, the CA3 region of the hippocampus was unaffected by sleep loss (Havekes et al., 2016). An overview of how sleep loss impacts spine density and different spine subtypes in hippocampal subregions is shown in Fig. 6. Together, the data from the present study and our previous work (Havekes et al., 2016) show that sleep loss has a regional effect at the level of dendritic structure. In line with these observations, other studies have reported changes in cortical spine numbers following SD (Diering et al., 2017; Li et al., 2017).

Upon closer inspection of the dendritic spine structure (e.g., spine shape, total length, head volume, head and neck diameter) one can identify a number of different categories including thin, stubby, mushroom, filopodia and branched spines (Hering and Sheng, 2001; Maiti, Manna, Ilavazhagan, Rossignol, & Dunbar, 2015; Sala and Segal, 2014; von Bohlen Und Halbach, 2009). After a brief period of SD, we observed a reduction of mainly the thin and branched spines in the DG. Branched spines have multiple heads originating from the same base spine and might reflect the beginning of a synapse duplication, leading to enhanced receptor turnover, which also can be observed during LTP (Hering and Sheng, 2001; Toni et al., 2001). Therefore, a reduction of branched spines might reflect a decrease in the potential to strengthen synapses, as is observed after sleep loss (Sala and Segal, 2014). Thin spines have a small head with a narrow neck and are suggested to be more flexible and react faster to stimuli (Sala and Segal, 2014). This is why thin spines have been considered to be 'learning spines' (Sala and Segal, 2014). A decrease of thin spines might therefore indicate a loss in the ability to react to afferent stimulations by both structural and functional plasticity such as the incorporation of new glutamate receptors in the spine head (Vogel-Ciernia et al., 2013). However, how sleep deprivation selectively targets these spine subtypes is not yet known. Future research is needed to elucidate if there are specific mechanisms affected in these spine subtypes, how these contribute to spine shrinkage, and ultimately to memory problems. Altogether, SD resulted in a decrease of branched spines in both blades of the DG, and a reduction of thin spines only in the inferior blade of the DG, supporting our finding that the inferior blade is more sensitive to sleep loss.

The observed higher sensitivity of the inferior blade compared to

the superior blade for SD could be related to the anatomical organization of the DG input. As mentioned above, a major input to the DG arises from the entorhinal cortex, via the perforant pathway (Amaral, Scharfman, & Lavenex, 2007, and references therein). The perforant pathway has two divisions, called the lateral and medial pathway, originating from the lateral and medial entorhinal cortex areas respectively. Both entorhinal projections terminate in the outer two-thirds of the molecular layer. Fibers from the lateral pathway target in the most superficial third of the molecular layer, whereas the fibers from the medial pathway terminate in the middle of the molecular layer. Given the observed laminar difference in spine changes after SD, this may suggest that the input of the medial entorhinal cortex rather than the lateral entorhinal cortex is affected by SD if the change is presynaptically driven. Both entorhinal areas receive input from different parts of the rhinal cortex. The perirhinal cortex preferentially projects to the lateral entorhinal cortex whereas the postrhinal cortex mainly innervates the medial entorhinal cortex (Naber, Caballero-Bleda, Jorritsma-Byham, & Witter, 1997). This may indicate that different types of information processed through the DG are differently affected by SD. Minor projections to the DG arise in various brain regions next to this major entorhinal cortex innervations. Associational, commissural, and hypothalamic afferents to the DG have been found to differ greatly in density between the inferior and superior blade, although functional differences between the blades are not well defined (Seress and Pokorny, 1981). Nevertheless, a striking anatomical difference between the blades is the difference in number of interneurons (e.g., basket cells). This number is 2–2.5 times higher in the superior blade (Seress and Pokorny, 1981). Associational projections from the pre- and parasubiculum terminate in the molecular layer between those of the lateral and medial perforant pathway, and presumably provides the DG with thalamic information (Kohler, 1985). Commissural fibers, arising from the hilar region, mainly terminate in the inner one-third of the molecular layer, just beside those of the medial perforant path (Tamamaki, 1999). Hypothalamic afferents end in the molecular layer as well as in the granular cell layer (Wyss, Swanson, & Cowan, 1979). Taken together, there seems to be no perfect match between the location of the spine impact of SD and the innervations patterns known to exist, neither within a blade or between the blades. Importantly, the vast majority of our knowledge on the anatomy of the DG, as briefly described above, is based on rat studies and for now we have to assume that the mouse anatomy resembles that of the rat. It cannot be excluded that the slightly different level of sensitivity for SD found in the two blades of the DG reflects a slight difference in intrinsic signaling processes within their DG granule cells or balances between granule cells and interneurons, irrespective of the origin of the presynaptic terminals.

Even though the molecular mechanisms underlying the effects of sleep loss are largely unknown, some molecular players have been identified. For example, SD decreases levels of brain-derived neurotrophic factor (BDNF), a molecule essential for memory retrieval and long-term potentiation, in the hippocampus, and importantly, specifically in the DG (Chauhan et al., 2016; Guzman-Marin et al., 2006; Zagaar et al., 2016). In this same special issue Delorme et al. show that ARC, a protein critical for synaptic plasticity like BDNF, is also reduced in the dentate gyrus after 3 h of SD (Delorme et al., *in press*). Furthermore, sleep loss impacts synaptic plasticity by attenuating cAMP levels in the hippocampus (Havekes and Abel, 2017; Luo, Phan, Yang, Garelick, & Storm, 2013; Vecsey et al., 2009). The reduction of cAMP after SD could be the result of altered expression levels of specific isoforms of the phosphodiesterase (PDE) family, a group of cAMP degrading enzymes that have been implicated in synaptic plasticity (Havekes et al., 2016; Houslay and Adams, 2003). Indeed, SD leads to an increase in the protein expression of PDE4A5 isoform (Vecsey et al., 2009), which is predominantly expressed in area CA1 and the DG (McPhee, Cochran, & Houslay, 2001). Sleep deprivation also reduces the phosphorylation of the cAMP response element-binding protein (pCREB),

which is associated with a decrease in synaptic plasticity in the hippocampus (Luo et al., 2013; Vecsey et al., 2009; Zagaar et al., 2016). Remarkably, the reduction of pCREB levels was also sub-region specific, as SD only affected pCREB levels in the CA1 and DG region of the hippocampus (Vecsey et al., 2009). A regional increase of PDE4A5 expression, leading to a decrease of cAMP and consequently resulting in the reduction of pCREB, may contribute to the observed region-specific changes at the level of the dendritic structure under conditions of sleep deprivation.

Chronic sleep loss inhibits hippocampal cell proliferation and neurogenesis, phenomena that occur almost exclusively in the dentate gyrus and are important for learning and memory (Meerlo, Mistlberger, Jacobs, Heller, & McGinty, 2009; Mueller, Meerlo, McGinty, & Mistlberger, 2015). The newborn cells in the DG that survive and eventually develop into neurons are incorporated in the granular cell layer and become functionally integrated in the hippocampal network (Meerlo et al., 2009). Although the available data indicate that cell proliferation and cell survival is only affected by chronic or prolonged sleep deprivation, not by brief sleep deprivation less than a day, it is not excluded that even a brief period of SD for 5–6 h affects spine formation and functional integration of the new cells. In the present study, discriminating between very young and adult neurons was difficult, as double labeling is impossible with Golgi impregnated sections. Therefore, whether an acute period of sleep loss causes a reduction of spines on existing cells or hampers the formation of spines on newborn cells is difficult to determine. However, we only included fully branched neurons, thereby avoiding the majority of newborn neurons. In addition, using different techniques such as Golgi, DiI-labeling can lead to the labeling of a different subset of neurons. Nevertheless, we have previously shown that spine loss was observed in neurons both using Golgi and the DiI-labeling technique (Havekes et al., 2016). In future studies, it would be interesting to use *thy1-eGFP* mice, in which enhanced green fluorescent protein (EGFP) is sparsely expressed in neurons, resulting in a bright Golgi-like staining. Brain sections of these mice are ideally suited for double labeling studies to assess the impact of SD on specific populations of neurons at different ages in the DG. In future studies, it would also be interesting to modulate for example cofilin or PDE4A5 in a Cre-dependent manner, specifically in the DG. This would allow to directly examine the effect of altered structural plasticity on certain memory tasks, such as pattern separation (McHugh et al., 2007). Unfortunately, to our knowledge it is technically impossible to specifically target neurons in just one blade of the DG. Therefore, the individual contribution of the inferior and superior cannot yet be determined.

It is important to note that it is unlikely that the observed results could have been caused by stress or other factors associated with the SD method. For example, while chronic stress might reduce spine density (Leuner and Shors, 2013), some studies suggest that acute stress does not affect or even increases spine density in the hippocampus (Shors, Chua, & Falduto, 2001). Furthermore, previous research has shown that prolonged SD attenuates DG-dependent mechanisms, such as neurogenesis, independent of stress hormones (Guzman-Marin, Bashir, Suntsova, Szymusiak, & McGinty, 2007; Meerlo et al., 2009; Mueller et al., 2008; Wadhwa et al., 2017). Also, REM-SD for 21 days using the multiple-platform method impairs hippocampal memory and reduces total volume of the DG (Noorafshan, Karimi, Kamali, Karbalay-Doust, & Nami, 2017).

Sleep loss is a growing health concern, especially in our 24/7 modern society, and entails severe consequences for brain function. Altogether, this study indicates that even a short period of sleep loss affects spine density in the DG. Results from these experiments further suggest that the observed changes were mainly derived from the changes in spine morphology of the inferior blade, rather than the superior blade of dentate granule cells. This is in line with recent findings showing that in children suffering from obstructive sleep apnea syndrome, the microstructure of the DG is disrupted which correlates with decreased learning capacity (Cha et al., 2017). Future studies are

needed to investigate the underlying mechanism by which sleep loss attenuates spine density in the dentate gyrus. One promising signaling mechanism is the cAMP-PKA-LIMK-cofilin pathway as we recently showed that this pathway mediates the spine loss in CA1 neurons (Havekes et al., 2016; Raven et al., 2017). In addition, it has to be clarified whether these reductions in spine density are a direct effect of sleep loss, or an indirect effect via phenomena such as hampered neurogenesis. Moreover, it would be of great value to examine whether preventing these specific changes in spine dynamics specifically in the DG would also avoid hippocampus-dependent memory deficits associated with sleep loss. Investigating the impact of sleep loss at the level of the neuronal cytoskeleton adds to the current understandings of how sleep loss results in memory deficits. In addition, it offers an interesting therapeutic target as preventing changes at the level of the dendritic tree might also prevent the negative consequences commonly associated with sleep loss.

Conflicts of interest

None.

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